THE MICROBE WILL HAVE THE LAST WORD

Prof Pieter Gouws
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ABOUT THE AUTHOR

Prof Pieter Gouws was born in 1961 and spent most of his early childhood on a farm in the Karoo. He matriculated from Randburg High School. He completed his BSc (Microbiology and Genetics) and BSc (Hons) in Microbiology at Stellenbosch University. He obtained his MSc in Microbiology from the University of Pretoria. In 1996 he received a British Council scholarship to do part of his PhD at the University of Nottingham in the United Kingdom. He obtained his PhD in Food Biotechnology from the University of the Western Cape (UWC) in 1999.

Prof Gouws joined UWC in 1989 as lecturer. He was promoted to senior lecturer in 2000, to associate professor in 2002 and to professor in 2010. He was also appointed as Extraordinary Professor in Biotechnology at UWC. During 2004, he spent some time as an international research fellow at the University of Nottingham, studying the effects of polymerase chain reaction inhibitors in complex food samples. He was appointed head of the Biotechnology Department from 2010 until his resignation from UWC in 2014.

Prof Gouws is also the South African representative on the International Commission of Food Microbiology and Hygiene (2008 to present) and member of the Food Legislation Action Group Specialist Working Group on Microbiological Standards, Department of Health, South African Government. He was a member of the Scientific Committee for the 23rd International Committee on Food Microbiology and Hygiene Symposium, 2012. He is also an advisor for the Namibian Government, Ministry of Agriculture, regarding food safety.

Prof Gouws and his research group were the first to develop a polymerase chain reaction-based method for the detection of *Salmonella* within 24 hours in complex food samples. They were the first to identify *Alicyclobacillus* in the South African fruit juice industry. They are in the forefront of using ultraviolet C technology as a nonthermal treatment for the inactivation of microorganisms in the food industry. In 2014, they also published a review of the influence of processing on the microbial risk associated with Rooibos (*Aspalathus linearis*) tea.

At present Prof Gouws is a professor in the Department of Food Science at Stellenbosch University, and his current research focuses on novel and innovative applications for the detection and control of unwanted bacteria in food.

He is married to Lizelle and has two daughters, Michelle and Lisa.

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THE OMNIPRESENT MICROBE

Louis Pasteur, the microbiologist who discovered the role of microbes in the process of fermentation and who played a major role in the field of food preservation, immunisation and vaccination, among other things, said, "It is the microbes that will have the last word."

Of course Pasteur did not actually use these exact words. He was French, so if he had said something to that effect at all, it would more likely have been, "Messieurs, c'est les microbes qui auront le dernier mot (Gentlemen, it is the microbes that will have the last word)." Pasteur's view was quite prescient and may well be proven to be correct within the foreseeable future and perhaps sooner than most of us would like to contemplate. It might just be possible that we are losing the war against microbes.

Microbes are everywhere. They are normally invisible, but they are abundant in the air that we breathe, in the food that we eat, in the water that we drink, on our skin, in our mouths and in our intestines. They ensure that the soil is fertile, they clean up the environment and they change and improve our food. Yet people are not normally aware that they exist; it is only when a disease breaks out or when food become spoilt that microbes are regarded as the invisible enemy.

In 1864 Pasteur discovered that heating beer and wine just enough to kill most of the bacteria that caused spoilage prevented these beverages from turning sour. Today the process of pasteurisation is used widely in the dairy and food industries for the preservation of the food that we consume. This is done on order to extend the shelf life and also to improve the safety of the food product (Hartmann, 1997).

Dealing with food safety problems is challenging and complex. Food safety failure is not a commercial option. Linking a product with a foodborne pathogen resulting in consumer illness is a catastrophic event for the food processor. Nearly 25% of the world's food supply is lost due to spoilage, that is, microbial spoilage. Problems with food quality and safety have existed for many centuries. Contaminated food results in major health problems in the word and leads to reduced economic productivity.

Microbial food poisoning is caused by a variety of microorganisms with various incubation periods and symptom duration (International Commission for the Microbiological Specifications of Foods , 2002). Organisms such as *Salmonella* and *E. coli* are well known to the general public, but in recent years newly emerging (and re-emerging) foodborne pathogens have been identified, specifically antimicrobial-resistant microorganisms whose incidence in humans has increased in the last decade and will increase in the near future. The emergence of these pathogens is due to a weakened or collapsed public health system due to economic problems, change in health policies and civil unrest. Poverty and uncontrolled urbanisation can play a major role. The degradation of water and food sources, ineffective disease control and the globalisation of food supply have all contributed to the increase of emerging foodborne pathogens.

Microorganisms have also evolved and have developed adaptation mechanisms to survive and persist in conditions otherwise unfavourable to microbial growth. They can exchange genetic material and acquire new genes (deoxyribonucleic acid [DNA]) in order to survive. Microorganisms can also exchange pathogenicity islands that encode for specific virulence factors (Forsythe, 2010).

With the globalisation of the food trade, food safety has become an international concern. More imported foods and food ingredients mean that we depend on food safety systems in other countries. Centralised production of foods means that when a problem occurs, it can lead to a widespread outbreak of disease.

Foodborne diseases are a widespread and a growing public health problem, both in developed and developing countries. The global incidence of foodborne diseases is difficult to estimate, but it has been reported that 1.8 million people die from diarrhoeal diseases per year (World Health Organization, 2007). A large proportion of these cases can be attributed to contamination of food and water. Additionally, diarrhoea is a major cause of malnutrition in infants and young children. In industrialised countries, the percentage of the population suffering from foodborne diseases each year has been reported to be up to 30%. In the United States of America, for example, around 76 million cases of foodborne diseases, resulting in 325 000 hospitalisations and 5 000 deaths, are estimated to occur each year (Mead *et al*., 1999).

Developing countries bear the brunt of the problem due to the presence of a wide range of foodborne diseases, including those caused by parasites. The high prevalence of diarrhoeal diseases in many developing countries suggests major underlying food safety problems.

Globalisation, centralisation and industrialisation have radically changed our food production and delivery systems in the past approximately 15 years. New scientific and detection methods for dealing with foodborne disease outbreaks are driving our understanding of risks and consequences. Increasing consumer awareness is driving the demand for food safety improvements.

It is therefore imperative to develop novel and innovative applications for the detection and control of unwanted bacteria in food. It is important to understand the biological and microbiological phenomena that are essential for the improvement of food safety and food quality. In order to accomplish this, a diverse range of research interests including food science, microbiology, biochemistry, molecular biology, microbial genomics, food safety and food technology needs to be employed to address the issues related to food safety.

MOLECULAR DETECTION OF PATHOGENS IN COMPLEX FOOD SAMPLES

DNA information for the detection and identification of selected foodborne bacteria is increasingly being used in the food industry (Gouws *et al*., 1998; Gouws and Liedemann, 2005; Gouws *et al*., 2005; Duvenage *et al*., 2007; Rip and Gouws, 2009) *Salmonella, Campylobacter and Listeria* are important human foodborne pathogens. Conventional methods for the detection of *Salmonella and Listeria* in foodstuffs are generally cumbersome and time consuming. Molecular methods have an enormous range of applications and have become a necessary tool in the detection of foodborne pathogens (Gouws and Liedemann, 2005). The polymerase chain reaction (PCR) is a method for the amplification of DNA sequences *in vitro*. However, without appropriate preparation of a test sample, it is possible that the food sample may drastically decrease the sensitivity of the detection.

Salmonella spp. is one of the most important groups of foodborne pathogens worldwide. Conventional methods for the detection of *Salmonella* spp. in foodstuffs are generally cumbersome and time consuming. Whereas various more rapid detection methods have been developed over the past few years, there is currently no reliable 24-hour detection method available. Gouws *et al*. (1998) report a reliable *Salmonella* PCR detection method yielding results within 24 hours. Chicken samples were pre-enriched in buffered peptone water for six hours. The DNA was extracted using phosphate-buffered saline and then heated at 95 °C for 10 minutes. The *Salmonella*-specific primers ST11 and ST15 were used to amplify a 429-bp region specific to all *Salmonella* spp. This approach proved to be sufficient for the reliable detection of *Salmonella* spp. from both artificially and naturally contaminated poultry samples. The characteristic 429-bp PCR product was obtained in artificially contaminated samples with a detection limit of 50 colony-forming units (CFU). A variety of chicken samples confirmed to harbour *Salmonella* spp. by conventional culture methods tested positive by our 24-hour procedure, whereas no detectable amplification product was detected in those samples testing negative by conventional culture methods. This method proved to be an excellent tool for the rapid and sensitive detection of *Salmonella* spp. from poultry samples using a specific primer set (ST11 and ST15) after only six hours of pre-enrichment.

The PCR is a highly sensitive, specific and rapid method for detection of bacteria in pure cultures, but the low concentration of pathogens and the presence of PCR inhibitors in complex food samples will reduce the amplification or even block DNA synthesis. The approach used was to target the hemolysin A of *Listeria monocytogenes* to obtain a 730-bp PCR product. Different pre-PCR treatments were developed to concentrate DNA or target cells and to counteract the effect of PCR inhibitors. Different DNA polymerases including *Taq* and *Tth* were evaluated, with different PCR buffers and different PCR facilitators (Gouws, 2005).

It was found that different enrichment broths did inhibit the PCR when different *Taq* DNA polymerases were used, but, surprisingly, when *Tth* DNA polymerase was used, there was no or little inhibition of the PCR. *Tth* polymerase from *Thermus thermophilus* was the most resilient, and it allowed the detection of only two CFU/g *Listeria monocytogenes* in food samples within 24 hours. Thus, the PCR-inhibiting effect of various components in biological samples can, to some extent, be eliminated by the choice of the appropriate thermostable DNA polymerase, and this factor needs to be considered when trying to develop PCR detection methods. What is unique is that it is the only technique that will pick up two *Listeria* cells after a short incubation period of six hours (Gouws, 2005).

To validate the accuracy of the PCR as a powerful molecular tool for detection, it is important that false negatives be distinguishable from true negative PCR

results. This can be overcome by the inclusion of an internal amplification control within the PCR to coamplify with the *Listeria monocytogenes* in order to identify false negatives (Rip and Gouws, 2009). The internal amplification control has to be incorporated without the loss of specificity and sensitivity.

ULTRAVIOLET C TECHNOLOGY IN THE FOOD INDUSTRY

Ultraviolet (UV) light has been used for surface treatment for disinfection. However, today there is a growing interest in using UV light for food preservation. UV wavelengths of 200–280 nm will inactivate bacteria and viruses, and liquids such as water and fruit juices have been successfully treated with UV light to reduce bacterial counts.

UV light is short-wave electromagnetic light in the electromagnetic radiation spectrum, with typical wavelengths of between 100 and 400 nm. On either side of the visible wavelengths in the spectrum are the invisible long waves such as radio waves and invisible short waves such as UV rays, X-rays and cosmic rays. UV light can be divided into four definitive subclasses, namely the vacuum UV range (100–200 nm), UV-A (315–400 nm), UV-B (280–315 nm) and UV-C (200–280 nm), as indicated in Figure 1. The latter is called the germicidal UV range as it will inactivate bacteria, viruses and protozoan microorganisms if such organisms are exposed to UV-C radiation. UV light in general is regarded as non-ionising radiation as it demonstrates low penetration power due to the inherent low energy of photons compared with ionising irradiation (Koutchma, 2009).

UV radiation is the most readily available source of radiation that is produced by arc discharges in mercury tubes. Radiative transfer is defined as the process during which light or other electromagnetic energy is transmitted from one form to another (i.e. through absorption, scattering, reflection etc.). The absorption of nonionising radiation induces excitation of atoms and/or molecules. Light is emitted from the gas discharge from UV lamps at wavelengths dependent upon its elemental composition and the excitation, ionisation and kinetic energy of those elements. Other types of radiation that can be used include X-rays, gamma radiation and beta radiation.

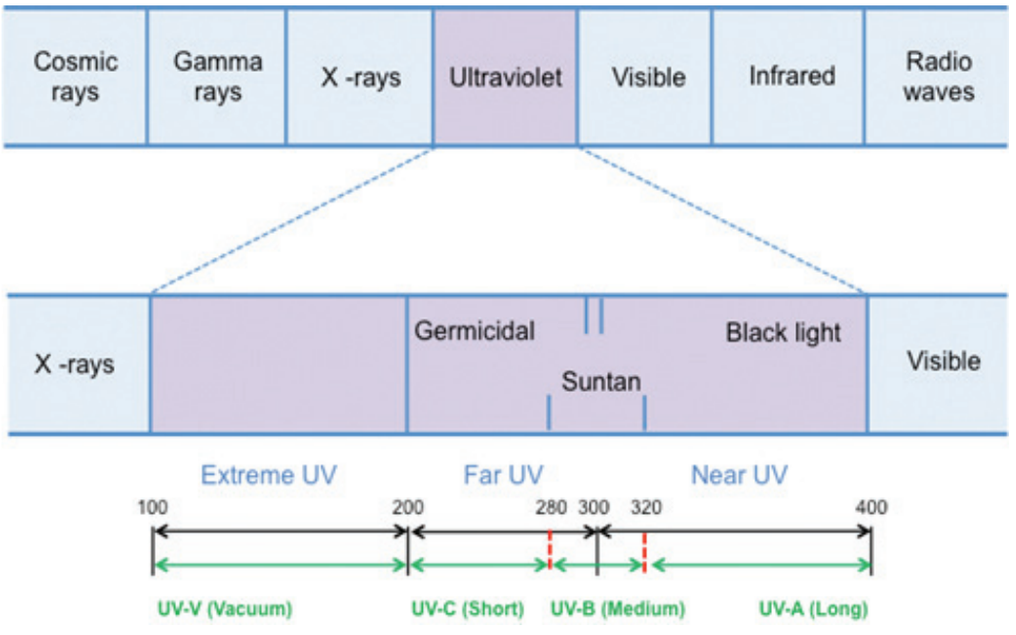

Figure 1: The electromagnetic spectrum (Adapted from Snowball and Hornsey, 1988)

UV radiation absorbed by microorganisms' DNA will impair cell proliferation and growth and will ultimately lead to cell death (Guerrero-Beltrán and Barbosa-Cánovas, 2004). UV light with a wavelength of 228–265 nm is the most deadly to microorganisms because this is the ideal absorption spectrum of the organisms' DNA. The germicidal effect at these wavelengths will be effective against microorganisms such as bacteria, viruses, protozoa, moulds, yeasts and algae. At these wavelengths, the DNA of the organism exposed to UV-C radiation will absorb the radiation, which will cause lethal mutations in the DNA. At wavelengths above 300 nm, the germicidal effect is limited and/or completely negated.

The germicidal effect of UV radiation is mainly a result of the dimerisation of the DNA of the microorganism being exposed to the UV radiation. As indicated in figures 2 and 3, the changes in the DNA structure are a result of cross-linking between thymine and/or cytosine (pyrimidine) nucleoside bases in the same DNA strand forming thymine dimers, which is proportional to the UV-C dose applied (Guerrero-Beltrán and Barbosa-Cánovas, 2004). The formation of thymine dimers in the DNA impairs DNA transcription and replication, therefore inactivating the microorganisms' cellular and physiological functions. Structural damage of cells exposed to UV radiation includes the rupturing of cell membranes, mechanical damage to cell walls and breaking of DNA strands (Cilliers *et al.*, 2014). Following UV radiation, lethal DNA lesions are scattered throughout the population of cells. The cells that are not able to repair the UV-induced damage to their DNA will not be able to replicate or transcribe further. Cells that were damaged but still survive usually undergo mutations. UV radiation can also cause a small amount of protein to denature. This happens when aromatic amino acids are cross-linked at their carbon-carbon double bonds. The denaturing of the proteins causes the cell membrane to be depolarised, and it also changes the ionic flow in the cell wall.

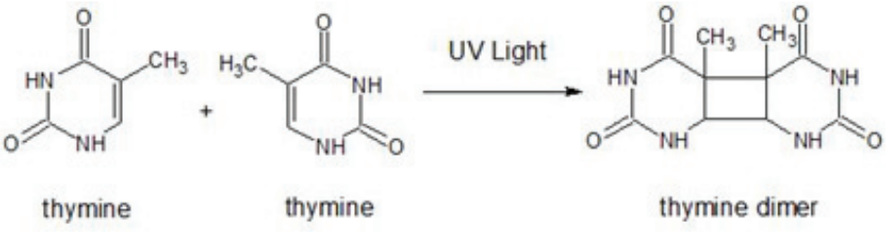

Figure 2: Cross-linking between thymine (pyrimidine) nucleoside bases to form a thymine dimer

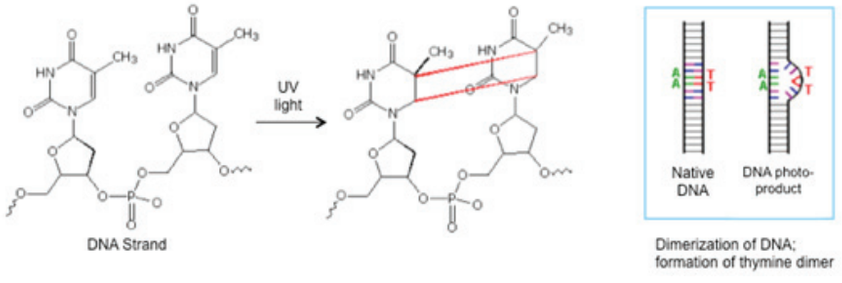

Figure 3: Dimerisation of DNA following exposure to UV light

Bacteria suspended in air are more sensitive to UV-C light than bacteria suspended in liquids due to the different penetration capacities of UV light through different physical media. The photoinactivating process by UV-C radiation is a physical method in which the energy is the germicidal medium. It does not produce undesirable by-products that could change the sensory characteristics (taste, odour and colour) in the final product. The use of UV light for sterilisation or disinfection also does not generate chemical residues. It is a dry, cold process that can be simple and effective at low cost in comparison with other sterilisation methods.

Another advantage when applying UV-C radiation is that it does not deliver residual radioactivity as ionising radiation (gamma radiation). However, UV-C light does not penetrate the target very deeply. Thus, it is more frequently used for surface sterilisation. Radiation is more effective on surfaces or transparent materials such as air, water and polyethylene. In addition, the germicidal effect is obtained only by applying direct UV-C light to the target. It is not effective in the shade, in pores or in orifices. The microbial reduction rate with UV-C light can be varied by applying low-intensity light for long periods or high-intensity light for short periods of time. Due to the wide variety of organisms, including strains, the dose levels required for disinfection can vary according to the final effect required for each food product (Koutchma, 2009).

The effect of UV radiation on microorganisms may vary from species to species and in the same species may depend on the strain, growth media, stage of culture, density of microorganisms and other characteristics, such as type and composition of the food (Reinemann *et al.*, 2006). Fungi and yeasts (large microorganisms) are more resistant during disinfection; however, high microbial levels should be taken into account when using UV-C for disinfection. The radiation absorbed by DNA may stop cell growth and lead to cell death. The UV-C light absorbed by DNA causes a physical shifting of electrons to induce splitting of the DNA bonds, delay of reproduction or cell death.

Microorganisms possess several recovery mechanisms to limit the effect of UV radiation and to promote cell survival. These processes present a potential hazard as sublethal stresses can cause the expression of cell repair systems. The exposure of a cell to UV light can induce enzymatic photo repair as well as the expression of excision repair genes that remove damaged DNA and replace it with functional DNA. For example, *E. coli* that can survive UV radiation usually has a gene called htpR that acts as a transcriptional regulator that is used to repair stress damage to the cell following UV radiation. Cells that are adapted to stress conditions are a particular hazard to the food and beverage industry since these organisms may survive UV radiation processes used as a stand-alone technology or in combination with other preservation technologies (hurdle technology). A further hazard is that repeated exposure of cells to sublethal UV radiation dosages can cause the formation of particularly resistant mutants that will be very difficult to eradicate using the preservation method of choice (Lado and Yousef, 2002).

In contrast with photoreactivation, dark repair in the microorganism does not need exposure to visible light in order to be activated. Under stress conditions, such as damage caused to the DNA of the cell following exposure to UV light, the RecA protein in the cell is activated. The activated RecA protein then cleaves the negative regulator, LexA protein, which represses the transcription of the genes involved in the SOS signal in the cell. As LexA is lowered, the SOS system in the cell is activated, which in turn is responsible for the dark repair mechanism. The SOS system is responsible for the activation of various genes involved in dark repair mechanisms and includes excision base pair repair, nucleotide excision repair, recombinant repair, mutagenic repair, phage control repair and multiplicity reactivation in viruses. As the repair mechanisms are activated, the level of the activated RecA protein is lowered and the concentration of the LexA protein is again increased to normal levels associated within the cell. The induction of the LexA gene in organisms is very specific and can be attenuated at UV-C dosage levels of between 100 and 600 J/m^2 (Jungfer *et al.*, 2008).

The commercial SurePure Turbulator™ system, designed and manufactured by SurePure AG, Switzerland, consists of 40 single 'turbulators' (SP-40) connected in series. A single UV turbulator applies a novel swirl-tube design promoting 'thin film' or 'surface refreshment' and consists of a stainless steel inlet and outlet chamber, with a corrugated spiral tube connecting the inlet and outlet chambers. The tangential inlet of the reactor creates a high velocity and turbulence in the inlet chamber and brings the whole volume of the liquid into contact with the UV photons. Inside the spiral tube, a low-pressure mercury UV-C lamp, protected by a quartz sleeve, is housed. The liquid flows in the gap between the corrugated spiral tube and the quartz sleeve at a minimum flow rate (F_r) of 4 000 l/h^{-1} (1.11 l/s^{-1}) with a Reynolds value (R_e) in excess of 6 000, indicating a turbulent flow pattern, as confirmed by Simmons *et al.* (2012) and the equipment manufacturer. In our study, the UV light intensity on

the surface of the quartz sleeve was 17.7 mW/cm^{-2} as measured by the lamp manufacturer, using calibrated radiometer. Figure 4 shows a cross-sectional view of a typical SurePure Turbulator™.

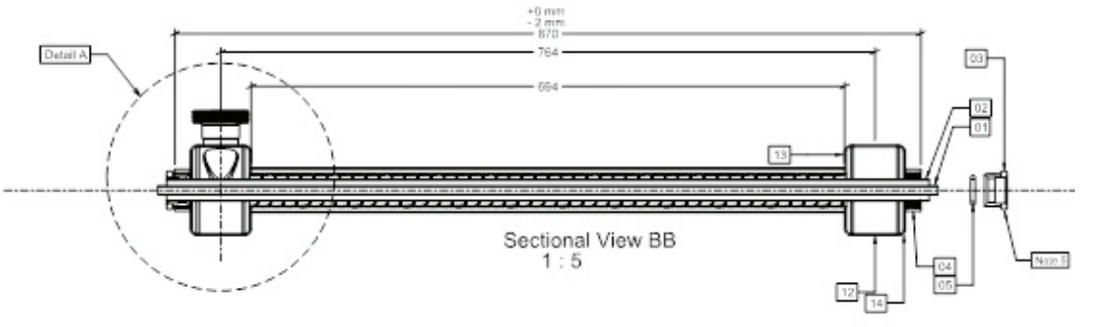

Figure 4: A cross-sectional view and outside view of the SurePure Turbulator™

The design specifically promotes four key points in order to achieve the desired germicidal efficiency in the liquid being treated, in this instance milk (Reinemann *et al.*, 2006). It is important that these critical operating parameters be adhered to when applying the SurePure technology using the current turbulator design specifications: Maintain the minimum flow rate of 3 800 l/h and minimum flow velocity of 1.5–3.0 m/s^{-1}. The UV-C intensity of all the lamps should be > 300 µW/cm^2 when measured at 1 m (> 25 UV-C watts). UV-C dosage applied can vary depending on the quality of the milk and the desired germicidal efficacy needed. Typical dosage values will range between 250 and 3 000 J/L^{-1}. Turbulent flow should be maintained through the SurePure system, and the Reynolds value should be > 4 500).

The SurePure Turbulator™ carries a patent registration (Patent number: US 6,916,452 B1) as per Annexure A. The US 6,916,452 B1 patent registration also forms the basis of various other patents registered to manufacturers that use the turbulator technology under license of SurePure AG, Switzerland.

Fruit juices (Keyser *et al.*, 2008) and milk (Van Wyk and Gouws, 2011) can be processed using UV-C light to reduce the number of microorganisms. A UV-C wavelength of 254 nm is used for disinfection and has a germicidal effect against microorganisms. This novel turbulent flow system was used for the treatment of apple juice, guava-and-pineapple juice, mango nectar, strawberry nectar and two different orange and tropical juices. In comparison to heat pasteurisation, juices treated with UV light did not change taste and colour profiles. UV dosage levels (J/L^{-1}) of 0, 230, 459, 689, 918, 1 148, 1 377, 1 607 and 2 066 were applied to the different juice products in order to reduce the microbial load to acceptable levels (see Figure 5). UV-C radiation was successfully applied to reduce the microbial load in the different single-strength fruit juices and nectars, but optimisation is essential for each juice treated. This novel UV radiation technology could be an alternative to thermal treatment or application of antimicrobial compounds (Keyser *et al.*, 2008).

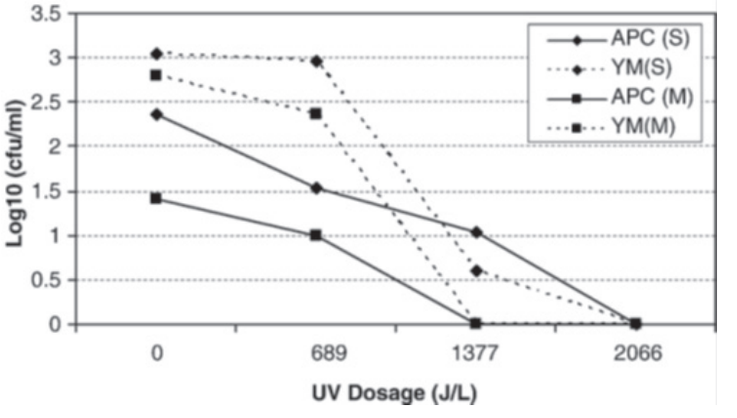

Figure 5: The log$_{10}$ reduction of the aerobic plate count (APC) bacteria (CFU/ml^{-1}) and yeasts and moulds (YM) in strawberry (S) and mango (M) nectar after exposure to different UV dosages (J/L^{-1})

The pilot scale system (20-l capacity) was used in order to determine the effect of UV radiation on the viability of two strains of *Mycobacterium avium* ssp. *paratuberculosis* (MAP) inoculated into milk. MAP in an ultra-heat-treated milk matrix was subjected to increasing doses of UV-C radiation from 0 to 1 836 mJ/ml^{-1}). Survival of MAP was monitored by culture on Herrold's egg yolk medium, Middlebrook 7H10 medium and the FASTPlaqueTB phage assay. Differences in sensitivity to UV treatment were observed between strains; however, at 1 000 mJ/ml^{-1}, a MAP kill rate of 0.1–0.6 log_{10} was achieved regardless of strain used or method employed to enumerate MAP. Although the inactivation trend was similar on the culture and phage assay, the former gave a consistently higher viable count. The use of UV radiation alone does not represent an alternative to current pasteurisation regimes for a large reduction in viable MAP in milk (Donaghy *et al.,* 2009).

Full-cream raw milk for the commercial production of Cheddar cheese was subjected to three treatments: high-temperature, short-time (HTST) pasteurisation (P), UV light treatment (UV) and a combination of HTST pasteurisation and UV light treatment (UVP). The Cheddar cheese produced was evaluated throughout a shelf life period of 12 months to establish differences in the ripening profile. During ripening, Cheddar cheese manufactured from UV-treated milk showed no statistically significant differences ($p > 0.05$) as far as the fatty acid profile and lipolysis were concerned when compared to the P and UVP treatments. Furthermore, a lower level of primary and secondary proteolysis was observed when compared to the P and UVP treatments, specifically after 12 months; however, the UV Cheddar cheese was rated as superior with regard to organoleptic properties as the P and UVP samples were perceived as bitter ($p < 0.05$) after 12 months. Turbulent UV light treatment systems could provide a viable alternative to traditional thermal processing of cheese milk (Cilliers *et al*., 2014). Treatment of milk with UV light demonstrated immense promise as a novel technology that could be used for the production of safe, value-added dairy products of good quality, such as Cheddar cheese.

THE INFLUENCE OF PROCESSING ON THE MICROBIAL RISK ASSOCIATED WITH ROOIBOS TEA

Rooibos tea, like other plant products, naturally contains a high microbial load (Du Plessis and Roos, 1986). Downstream processing of these products usually helps in reducing any contaminants present. Due to the delicate flavour properties and nature of Rooibos, gentle processing techniques are necessary for the production of good-quality tea (Joubert *et al.,* 2008; Gouws *et al.,* 2014). However, these techniques have a major influence on the microbiological status of the product. The presence of *Salmonella* in Rooibos is poorly understood (Swanepoel, 1987). The ubiquitous distribution of *Salmonella* in the natural environment and its prevalence in the global food chain, the physiological adaptability thereof, the virulence of the bacterial pathogen and its serious economic impact on the food industry emphasise the need for continued awareness and stringent controls at all levels of food production. With the advances of technology and information at hand, the processing of Rooibos needs to be re-evaluated. Since the delicate nature of Rooibos prohibits the use of harsh methods to control *Salmonella*, alternative methods to steam pasteurisation of Rooibos show great potential to control *Salmonella* in a fast, efficient and cost-effective manner. These alternative methods will significantly improve the microbiological quality of Rooibos and provide a product that is safe to consumers.

The fermentation process provides optimal conditions for the growth of microorganisms that would yield high bacterial counts. These conditions allow for the proliferation of pathogens such as *Salmonella*, which is unwanted in any food product (Gouws *et. al*., 2014). In this study, 35% of Rooibos samples tested before pasteurisation were positive for *Salmonella.* Even prior to pasteurisation, the presence of *Salmonella* indicated that the processing and fermentation allowed for *Salmonella* to be present. Both the total viable count and the lactic acid bacterial count were very high during fermentation. It was, however, concluded by molecular fingerprinting enterobacterial repetitive intergenic consensus sequence-based (ERIC) PCR that these *Salmonella* strains were not directly linked to one another and thus did not originate from a single source. *Salmonella* originated from the various farms and not from the processing environment. The lactic acid bacterial had no direct influence on the presence of *Salmonella* in Rooibos. Therefore, better processing practices must be incorporated during harvesting as well as during transportation of Rooibos. None of the Rooibos samples tested positive for *Salmonella* after pasteurisation (steam pasteurisation at 135 °C for 1.5 min – product temperature of 94 °C).

The presence of *Salmonella* in Rooibos during fermentation requires that the quality control measures of the Rooibos industry be upgraded and re-evaluated as *Salmonella* infection allows for the proliferation of this

pathogen, which may result in problems arising during the further processing of this food product. Control of *Salmonella* before Rooibos is pasteurised will prove to be beneficial. Control of *Salmonella* during fermentation by selected lactic acid bacteria may just prove to be beneficial. Steam pasteurisation at 135 °C for 1.5 minutes with the product temperature reaching a minimum of 94 °C has been shown to eliminate *Salmonella* from Rooibos (Gouws *et al.*, 2014).

With the global trend steering towards healthier food choices, it is not unlikely that the demand for Rooibos will reach greater heights. The high consumption of Rooibos tea worldwide therefore makes heavy demands on the microbiological quality and safety of the product; however, little is known about its microbiological status. To date, the scientific literature available has focused on the quality and health benefits of Rooibos rather than its safety.

The fact that the conventional processing of Rooibos can potentially promote the growth of *Salmonella* and other microbial contaminants is of great concern to human health. Further investigation is therefore needed to isolate the sources of *Salmonella* contamination within the processing of Rooibos to be able to implement preventative and control measures to ensure the safety of consumers. The microbiological quality of the tea will also have severe implications for the tea export industry and manufacturers if the presence of *Salmonella* is not dealt with effectively.

With the advances in technology and the information at hand, the processing of Rooibos needs to be re-evaluated. Since the delicate nature of Rooibos prohibits the use of harsh methods to control *Salmonella*, alternative methods to steam pasteurisation of Rooibos show great potential to control *Salmonella* in a fast, efficient and cost-effective manner. These alternative methods will significantly improve the microbiological quality of Rooibos and provide a product that is safe for consumers (Gouws *et al.*, 2014).

The advances in Rooibos production are inevitably hindered by the lack of information available, which is both minimal and dated. Extensive studies are thus needed to investigate and isolate the sources of microbial contamination and to potentially improve on the processing of Rooibos, thereby reducing the risks and health concerns involved.

THE USE OF ANTIBIOTICS IN FOOD

We totally depend on antibiotics for the treatment of infectious diseases, and they should never be regarded as mere commodities to be used in food and agriculture. Notwithstanding the good intentions to control antibiotic usage, there is little doubt that the situation with respect to antibiotic resistance of food-related and disease bacteria is not looking good. Poor medical and veterinary antibiotic use and the lack of regard for the practice of infection prevention and control have left South Africa in a situation that most of the bacterial infections resulting from food are untreatable (Manie *et al.*, 1998; Manie *et al.*, 1999; Gouws and Brozel, 2000; Mokgatla *et al.*, 2002; Fielding *et al.*, 2012).

After more than 70 years of widespread agricultural use of antibiotics, evolution of disease-causing microbes has resulted in many antibiotics losing their effectiveness. As microbes evolve, they adapt to their environment. If something prevents them from growing and spreading, such as an antimicrobial, they evolve new mechanisms to resist the antibiotics by changing their genetic structure. Changing the genetic structure ensures that the offspring of the resistant microbes also are resistant (Capita and Alonso-Calleja, 2013).

Antibiotic-resistant bacteria were first described in the 1940s and with new antimicrobials that are coming onto the market have led to the problem of antibiotic resistance fast escalating into a global health crisis with specific concern about foodborne pathogens. Although the selective pressure exerted by the abuse of antibiotics has been deemed the major factor in the emergence of bacterial resistance to these antimicrobials, concerns about the role of the food industry have been growing in recent years. The selective pressure exerted by the use of antibiotics in primary food production is the main driving force behind the selection and spread of antimicrobial resistance throughout the food chain (Newell *et al.*, 2010).

Genetically modified crops with antibiotic resistance marker genes, microorganisms added intentionally or unintentionally to the food chain with potentially transferable antimicrobial resistance genes and food processing technologies used at sublethal doses are also issues for concern (Gay and Gillespie, 2005).

Microbes, such as bacteria, viruses and fungi, are living organisms that evolve over time. Their primary function is to reproduce, thrive and spread quickly and efficiently. Therefore, microbes adapt to their environments and change in ways that ensure their survival. If something prevents their ability to grow, such as an antimicrobial, genetic changes can occur that enable the microbe to survive. There are several ways in which this happens.

In the presence of an antimicrobial, microbes are either killed or, if they carry resistance genes, survive. These survivors will replicate, and their progeny will quickly become the dominant type throughout the microbial population. Most microbes reproduce by dividing every few hours, allowing them to evolve rapidly and adapt quickly to new environmental conditions. During replication, mutations arise and some of these mutations may help an individual microbe survive exposure to an antimicrobial. Microbes also may acquire genes from each other, including genes that make the microbe drug resistant. The use of antibiotics, even when they are used appropriately, creates a selective pressure for resistant organisms. However, there are additional societal pressures that act to accelerate the increase of antimicrobial resistance. Selection of resistant microorganisms is exacerbated by inappropriate use of antibiotics. Sometimes healthcare providers will prescribe antibiotics inappropriately to an insistent patient who has a viral infection or an as-yet undiagnosed condition. These situations contribute to selective pressure and accelerate antimicrobial resistance.

During food production, a variety of antimicrobials are used to improve food safety and quality. Antibiotics have been used in animal production to control and prevent diseases and to improve weight gain. The emergence of foodborne pathogens with multiple antibiotic resistances has led to various studies indicating that the use of antibiotics in animal husbandry leads to new resistance patterns in foodborne bacteria. The concern has been that foodborne pathogens will select for resistance from these antibiotics and result in an infection that will be untreatable. A still bigger concern is that the normal gut bacteria might pick up resistance genes resulting in untreatable infections later on. Inappropriate use of antibiotics is a critical factor in selecting resistance. The abuse of broad-spectrum antibiotics in high-density animal production without proper diagnosis of illness increases the chances of bacteria developing resistance.

Of growing concern is the increasing number of pathogenic bacteria resistant to antibiotics in the environment. Antibiotics are often excreted unchanged and create an opportunity for resistance selection (Arias *et al*., 2009). It has been found that flies collected from areas near abattoirs have a resistance pattern similar to the types of antibiotics used. It is thus inevitable that antibiotic-resistant bacteria will make it through the food chain and eventually will reach the consumer (Graham *et al*., 2009).

Antimicrobial use in agriculture can also compromise human therapies when bacteria develop cross-resistance. This happens when their resistance to one drug also makes them resistant to other related drugs. This has happened in Europe with vancomycin, one of the drugs of last resort for treating certain life-threatening infections. Data suggest that rising levels of vancomycin-resistant bacteria in hospitals may have resulted from the use in agriculture of avoparcin, a drug chemically related to vancomycin. Because avoparcin and vancomycin are similar in structure, bacteria resistant to avoparcin are resistant to vancomycin as well.

Antimicrobial resistance makes it harder to eliminate infections from the body. As a result of a microbe's ability to survive in the presence of antibiotics, some infectious diseases are now more difficult to treat than they were just a few decades ago. While the links between animal agriculture and human disease are complicated and in need of additional study, the evidence is strong enough for scientists and public health organisations to call for reduced use of antibiotics in agriculture.

In a recent food-related outbreak in Germany, researchers noted that the *E. coli* O104 strain carried a unique set of virulence and antibiotic-resistant factors, making it distinct from other strains of *E. coli*. The researchers discovered that the Shiga toxin was produced more abundantly when certain antibiotics were used, meaning that patients' symptoms worsened when they were treated with antibiotics. Doctors then stopped using antibiotics to treat infected patients. It is alarming to think that due to the use of antibiotics, one's intestinal flora might be disrupted, resulting in an increased risk of becoming infected with one's own intestinal pathogens resistant to an antibiotic (Muniesa *et al*., 2012).

Although reducing or eliminating nontherapeutic uses of antibiotics is a straightforward solution to the problem of resistance, it will be difficult to implement. The most sensible approach is to identify and reduce nonessential uses of antibiotics and reserve as many of these drugs as possible for wise use in human and veterinary medicine. Obvious nonessential uses, such as nontherapeutic use in livestock operations, should be the first target in the effort to save antibiotics.

Although not new, the problem of antibiotic resistance will increase and the food industry needs to play its part in producing safe food. It is now time to phase out the use of certain antibiotics for enhanced food production.

CONCLUSION

Foodborne diseases caused by a variety of microorganisms can be expected to continue to be a major challenge to the food industry. As the structure of the food industry has changed, so has the microorganism evolved to adapt to the new environment. This could lead to the emergence of new hazards to food safety.

Globalisation, centralisation and industrialisation have radically changed our food production and delivery systems. It is therefore imperative to develop novel and innovative applications for the detection and control of unwanted bacteria in food. It is important to understand the biological and microbiological phenomena that are essential for the improvement of food safety and food quality. In order to accomplish this, a diverse range of research interests including food science, microbiology, biochemistry, molecular biology, microbial genomics, food safety and food technology needs to be employed to address the issues related to food safety.

Thus the search for greater understanding of the activities of microbes in our food supplies and our environment continues.

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